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ULTRASTRUCTURAL CYTOCHEMISTRY OF THE ALDEHYDE-SENSITIVE ENZYMES

LOCALIZATION OF GLUCOSE 6-PHOSPHATASE ACTIVITY IN MOUSE HEPATOCYTES

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It has been said that the best tissue preservation at the electron microscopic level was obtained by fixation of tissue blocks promptly after sacrification of animals (1). Therefore, in cytochemical studies with the electron microscope, prefixation of tissue in glutaraldehyde or formaldehyde has been usually performed prior to incubation in reaction medium. However, as the *aldehyde-sensitive enzymes* are rapidly inactivated during aldehyde fixation, the ultrastructural demonstration of these enzymes is difficult (2) (3) (4).

The prefixation procedures in common use for the ultrastructural cytochemistry is *immersion fixation of tissue blocks* and *vascular perfusion fixation* (4). In immersion fixation of tissue blocks, the activities of the aldehyde-sensitive enzymes are markedly reduced when the fixatives penetrate throughout the tissue blocks. Therefore, this procedure is not suitable for the demonstration of the aldehyde-sensitive enzymes. In vascular perfusion fixation, the rapid penetration of the fixatives throughout the tissue after very short periods of time seems to preserve a considerable amount of the enzyme activities. At present this procedure has been accepted as the most reliable prefixation method for the demonstration of glucose 6-phosphatase (G-6-Pase) that is one of the aldehyde sensitive enzymes (4). However, in very small animals, embryos and adult fowls, the vascular perfusion is extremely difficult to perform.

I have previously observed that immersion of cryostat sections of the liver into glutaraldehyde for short periods of time caused only a slight reduction in G-6-Pase activity (5). This led to a development of a prefixation technique for the ultrastructural demonstration of G-6-Pase in rat liver (6). With application of this technique, the ultrastructural localization of G-6-Pase was investigated in mouse hepatocytes.

MATERIAL AND METHODS

Male adult DDD mice were used. The experimental methods were the same as those described previously (6).

The sections were cut with glass knives on LKB ultratome, stained with uranyl acetate and lead hydroxide and examined in a JEM-7A electron microscope.

RESULTS AND DISCUSSION

The reaction product was present in the endoplasmic reticulum and nuclear envelope as in rat hepatocytes (2) (4) (6). The Golgi apparatus, lysosomes, microbodies, mitochondria, plasma membranes and nuclei were nonreactive.

In the endoplasmic reticulum, the deposits of final product were seen in all of both smooth and rough surfaced types. The reaction product usually filled the cisternal spaces, but was also found on or immediately adjacent to the inner surface of the membranes in somewhat dilated forms of the endoplasmic reticulum. In the nuclear envelope the reaction product was located in the double perinuclear space.

Treatment of the control sections in 0.1 M acetate buffer, pH 5.0 at 37°C for 15 minutes caused a total loss of the final product.

Preincubation of the sections for 15 minutes in 0.25 M sucrose containing 1 mM Cu^{++}, 10 mM Zn^{++}, 10 m M F^{-} or 10 m M CN^{-} and the addition of these ions to the incubation medium resulted in a complete absence of the reaction product. Incubation of the sections in medium containing equal moles of β-glycerophosphate or fructose-1,6-diphosphate in place of the usual substrate in the Wachstein-Meisel medium abolished total reaction. These control experiments indicate that the reaction product in the endoplasmic reticulum and nuclear envelope is related to G-6-Pase activity.

In the present study the preservation of fine structure was satisfactory in spite of the freezing and thawing of fresh tissue. Therefore, the present method seems useful for the fine structural demonstration of the aldehyde-sensitive enzymes in the small animals and embryos, and further because of the simplicity of the technique also in those cases in which the vascular perfusion is possible.

The cytochemical localization of G-6-Pase activity with electron microscopy has been reported in rat hepatocyte (2) (4) (6) (7) (8), jejunal epithelial cell of mouse (9), B cell of rat pancreas (10), endometrial epithelial cell of human (11). These works have revealed the common presence of the enzyme activity in the endoplasmic reticulum and nuclear envelope to these cells. The hepatic enzyme is concerned in gluconeogenesis (12). The presence of the enzyme activity in the endoplasmic reticulum indicate that the final step of gluconeogenesis is carried out in the endoplasmic reticulum and glucose produced by the hydrolysis of glucose 6-phosphate is released into the cisternal space. The released glucose moves from here to the cell exterior by unknown process. The specific metabolic functions of the jejunal enzyme are not known except for a possible role in the conversion of fructose to glucose (13). The role of the enzyme in the pancreas and endometrium is also unknown (10) (11). Thus, the roles of the enzyme in these tissues appear not to be identical. Nevertheless, the localization pattern of the enzyme activity in the endoplasmic reticulum and nuclear envelope is common to these tissues. These facts are of interest in connection with the metabolic functions of the enzyme.

Summary

Ultrastructural localization of glucose 6-phosphatase activity was investigated in mouse hepatocytes. The reaction product for the enzyme activity was present in the endoplasmic reticulum and nuclear envelope, as in rat hepatocytes. The physiological significance of the presence of the enzyme in the endoplasmic reticulum is discussed.

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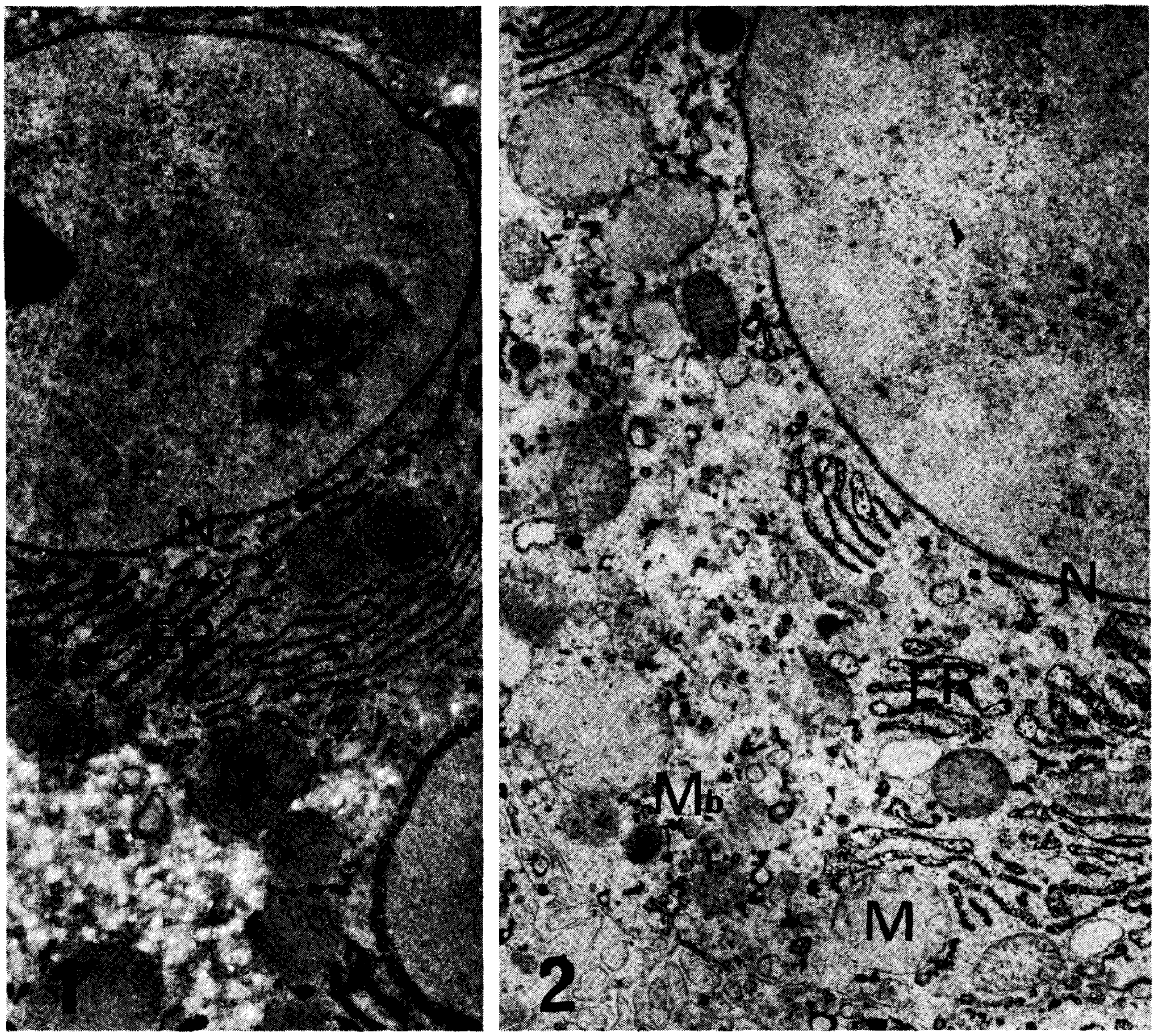

Fig. 1. Mouse liver sectioned at 20 μ in a cryostat, fixed for 5 minutes at 4°C in 2% glutaraldehyde, incubated for 15 minutes in Wachstein-Meisel medium for the demonstration of G-6-Pase and postfixed in OsO_4. The reaction product for the enzyme activity is located in the endoplasmic reticulum (ER) and nuclear envelope (N). No reaction is noted in the mitochondria (M). Poststained with uranyl acetate and lead hydroxide. ×10,000.

Fig. 2. Mouse liver treated as in Fig. 1. The reaction product for G-6-Pase activity is present in the endoplasmic reticulum (ER) and nuclear envelope (N). The mitochondria (M), mitcrobodies (Mb) and plasma membrane contain no deposition of final product. ×10,000.